DATA EVALUATION RECORD

STUDY 7a

CHEM 112600 Prohexadione Calcium §163-1
CAS No. 127277-53-6
FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 44457788
O'Connor, J. 1992. Determination of the seepage behavior of BX-112 by soil column studies in European soils (aged test). LSR Report No.: 92/0142. BASF Reg. Document No.: 92/11982. Unpublished study performed by Life Science Research Ltd., Suffolk, ENGLAND; and submitted by BASF Corporation, Research Triangle Park, NC.

DIRECT REVIEW TIME = 55 Hours

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CONCLUSIONS

Mobility - Leaching & Adsorption/Desorption

1. This study is not scientifically valid and not acceptable for the partial fulfilment of the data requirement for soil mobility of prohexadione calcium(column leaching). Based on column leaching studies, cyclohexene ring-labeled [3,5-^{14}C]prohexadione calcium, applied at a nominal concentration of 0.1 mg/kg and aged in loam and loamy sandy soils adjusted to 40% of the maximum soil water-holding capacity and incubated at 20 ± 1 °C in darkness, appeared to have low mobility in the loam and loamy sand soil columns which were leached with ca. 8 inches of distilled water over a period of two days. The test design and analytical method were inadequate to accurately determine the mobility of the test compound and its degradates.

2. This study does not meet Subdivision N Guidelines for the partial fulfillment of EPA data requirements on soil mobility (column leaching) for the following reasons:

   (i) the elution volume was not equivalent to 20 inches;

   (ii) the leaching solution was not 0.01-0.02 *N* $CaCl_2$ solution;

   (iii) residues were not characterized after aging and prior to leaching;

   (iv) leaching data were not reported adequately;

   (v) the soil treatment rate was lower than the lowest proposed application rate; and

   (vi) only foreign soils (two) were utilized in the study.

3. To satisfy the Subdivision N data requirement the Registrant should repeat the aged leaching study considering the above comments elaborated in The Reviewers Comments section of this review.

METHODOLOGY

Test Substance: Cyclohexene ring-labeled [3,5-^{14}C]prohexadione calcium, calcium 3-oxido-4-propionyl-5-oxo-3-cyclohexene-carboxylate; radiochemical purity 98.4%, specific activity 3.00 Mbq/mg; Lot No. CP-1068.

Reference Substance: [C^{14}]-atrazine, radiochemical purity 98.7%, specific activity 4.28 MBq/mg.

Soil Tested: Two European soils: a loam soil (Hallsworth, England; 31% sand, 48% silt, 21% clay, 3.4% organic carbon, pH 6.2, CEC 26.4 meq/100 g) and a loamy sand soil (Newport, England; 80% sand, 12% silt, 8% clay, 0.6% organic carbon, pH 6.9, CEC 6.4 meq/100g); stored at 4 °C for no longer than 3 months. The study author classified the loam and the loamy sand soils (USDA classification) as a clay loam soil and a sandy loam soil, respectively (see attachment p. 20).

Test Design: Duplicate samples (100 g) of sieved (2 mm) loam and loamy sand soils were placed in biometer flasks and adjusted to 40% of the maximum water-holding capacity. Samples were pre-incubated at 20 ± 1 °C in darkness for 14 days. The soil microbial biomass was estimated using the chloroform fumigation-incubation method. Results indicated that the soils were viable, the loam soil microbial biomass was 29.19 mg C/ 100 g soil and the loamy sand soil was 29.77 mg C/ 100 g soil.

Pre-incubated soil samples were treated with cyclohexene-ring labeled [3,5-^{14}C]prohexadione calcium, dissolved in distilled water, at a nominal application rate of 0.1 mg/kg. The treated soil was aerobically aged in an incubator for 13 hours in darkness at 20 ± 1 °C (p. 27). Humid air was passed through the system and into CO_2 (mono-ethanolamine:water; 20:80, v:v), and organic volatile (2-ethoxyethanol) traps. Soil samples were not analyzed following the aging period.

To determine pesticide mobility, 8 glass columns (5-cm internal diameter x 48 cm length) equipped with conical bottoms (filled with siliceous sand) were packed (while agitating) to a depth of 28 cm with untreated, sieved (2 mm) loam and loamy sand soil; columns were saturated with distilled water (p. 28). The aged treated soil was added on top of the treated soil columns as a layer of approximately 2 cm (as the 28- to 30-cm layer); duplicate columns and control columns were utilized for each soil. Two additional soil columns were treated with [^{14}C]-atrazine (29.4 kBq per column) for comparison. The [^{14}C]-atrazine treated columns and control columns were filled to depth of 30 cm with each soil type. CO_2 traps (1 M KOH and mono-ethanolamine:water; 20:80, v:v) were connected to the top of each column and to the leachate collection flasks (Figure 1, p. 43). At room temperature the columns were leached with 393 mL (20 cm) of distilled water over a period of 48 hours (*p. 29; see Comment #1*); the leachate was collected in 100-mL fractions.

Sample Analysis: Aliquots of the leachate fractions were analyzed for total radioactivity by LSC (p. 30); limits of detection and quantitation were not reported. Leachate fractions were placed in sealed glass containers connected to two CO_2 (0.1 M NaOH) traps in sequence. Fractions were acidified with 10 *N* sulfuric acid, aerated for 3 hours, and treated with nonradiolabeled 3,5-dioxo-4-propionyl-cyclohexane-1-carboxylic acid. The fractions were extracted three times with chloroform; the method of extraction was unspecified. The organic and aqueous phases were brought to known volume and duplicate aliquots were analyzed for total radioactivity by LSC.

The [^{14}C]-BX 112 fortified soil columns were divided into five 6-cm sections (p. 29). Each section was washed with acetone into a sealed glass container connected to two CO_2 (0.1 M NaOH) traps in sequence. The soil samples were acidified with 1 *N* sulfuric acid and aerated for four hours. Samples were extracted by shaking with acetone and filtered. The combined filtrates were concentrated under vacuum and treated with nonradiolabeled 3,5-dioxo-4-propionyl-cyclohexane-1-carboxylic acid (KI-2817). The filtrate was partitioned three times with chloroform. The organic and aqueous phases were brought to volume and duplicate aliquots were analyzed for total radioactivity by LSC. Duplicate subsamples of the post-extracted soil were analyzed for total radioactivity by LSC following combustion (p. 31).

Each [^{14}C]-atrazine fortified soil was shaken with acetone and 1 *N* sulfuric acid, filtered, and filtrate was rinsed with acetone. Combined filtrate was concentrated to 40 ml and three times extracted with chloroform. Both organic and aqueous phases were brought up to known volumes and the radioactivity was determined via LSC. Extracted soil was combusted and analyzed for radioactivity.

Also, trap contents were analyzed for radioactivity. To characterize [^{14}C]-residues, leachate and soil extracts containing >0.001 ppm were analyzed by TLC (p. 31). Aliquots were analyzed using two-dimensional TLC on silica gel plates developed perpendicularly with benzene:methanol:acetic acid (45:8:4, v:v:v) followed by diisopropylether:formic acid:water (90:7:3, v:v:v); limits of detection and quantitation were not reported. Samples were co-chromatographed with nonradiolabeled reference standards which were visualized under UV (unspecified wavelength) light. Radioactivity was detected by linear analyzer (pp. 30, 31).

Sample Storage: Prior to analysis eluate samples were stored in a refrigerator at +4 °C, whereas soil samples were stored in a freezer at approximately -20 °C. The lag time from the treatment time to the completion of analysis was from 2 (loam soil - fortified columns) to 10 (loamy sand soil - fortified columns) months.

THE AUTHOR'S DATA SUMMARY

Based on column leaching studies cyclohexene ring-labeled [3,5-^{14}C]prohexadione calcium applied at a nominal concentration of 0.1 mg/kg and aged (13 hours) in loam and loamy sand soils adjusted to 40% of the maximum soil water-holding capacity and incubated at 20 ± 1 °C in darkness, appeared to have low mobility in the loam and loamy sand soil columns which were leached over a period of two days.

Table 1 and 2 present the total radioactivity distributions in the column soil sections, leachate (i.e., aqueous and organic extract, extracted eluent fraction), and traps, for the loam and loamy sand soil, respectively. Table 3 and 4 present the percent of radioactivity recovered in each soil segment of the BX-112 treated loam soil (Table 3) and loamy sand soil (Table 4), and atrazine columns.

Following the aging period radiolabeled $^{14}CO_2$ accounted for the means of 33.5% and 39.5% of the applied radioactivity in the loam and loamy sand soil, respectively. Based on LSC analysis, most of the total radioactive residues retained in the soil column following leaching were present in the 24- to 30-cm depth (the top layer, including application layer) and were 38.5% and 36.6% of the applied radioactivity for the loam soil and loamy sand soil, respectively. In the 18- to 24-cm depth (second layer from the top), 9.2% and 1.6% of the applied was present in the loam and loamy sand soil, respectively, and next layer even less of the residues was present (loam: avg. 3.6%; loamy sand: avg. 1.3%). In the remaining layers, sand (bottom), and wash, ≤1.1% of the applied radioactivity was present in both soils. Radiolabeled $^{14}CO_2$ collected from the loam soil in the traps at the top of the column and the leachate collection flasks accounted for means of 12.5% and 0.1% of the applied radioactivity, respectively, from the loamy sand soil they were 11.8% and 2.1% of the applied, respectively. Radiolabeled $^{14}CO_2$ collected in the traps during soil extraction accounted for a mean of 2.5% of the applied for loam soil and 4.6% and 1.0% of applied for loamy sand soil.

For both soil types data varied between replicate columns. Following column leaching, the material balance (based on LSC analysis) was 94.4-107.2% and 96.7%-102.3% of the applied radioactivity for the two loam and loamy sand soils, respectively (Table 1, p. 56; Table 2, p. 57).

THE AUTHOR'S COMMENTS

1. This study is not scientifically valid and not acceptable for the partial fulfilment of the data requirement for soil mobility of prohexadione calcium (column leaching). The study author stated that the depth of leaching in the two soils (the loam and loamy sand soil) indicated that the total residues were less mobile than atrazine which, according to Helling (1971), has medium mobility. The columns were leached with 393 mL of distilled (deionized) water (p. 29) for two days. Mobility determinations were made for parent compound plus degradates, as individual data were not reported in a valid manner. The test design and analytical method were inadequate to accurately determine the mobility of the test compound and its degradates for the following reasons:

   A. The [^{14}C]residues in the aged soil were not characterized after aging/prior to leaching. The total radioactivity and the percentage of the applied present as parent could not be determined. It could not be determined whether a sufficient amount of parent compound remained for the determination of soil mobility (≥50% of the applied radioactivity) following the 13-hour aging period. The study author stated that the soils were aged for 13 hours, which was approximately one half-life of the test compound based on the results of one, not submitted, aerobic metabolism study (p. 27; LSR Report No: 92/KCI118/0272). The reviewer noted that an aerobic metabolism study (MRID 44457785) utilizing loamy sand soil from Holly Springs, NC, was submitted. In the present study, the soils utilized to determine mobility were obtained from England.

B. Mobility determinations were made for total residues (parent compound plus degradates), as individual data were incomplete and were not reported in a valid manner. Residue data (based on TLC analysis) were only reported for the top one (loamy sand) or two (loam) sections of the soil columns and the percentage data appeared to be reported as fractions of the extractable radioactivity for each soil section rather than as percentages of the applied (Table 13, p. 68).

C. The soil columns were not sufficiently eluted. The elution volume was ca. 8 inches (393 mL) while it was supposed to be an equivalent of 20 inches which is 997 ml {i.e.; $((\pi^*$ (column diameter)$^2)/4)^*$ 20 inches = (3.1416*5*5)/4 *50.8=997 ml)}. Only a sufficient elution volume can provide downward movement of the parent and its degradates through the soil column.

D. The leaching solution was not 0.01-0.02 *N* $CaCl_2$ solution but distilled water. The use of distilled water could cause soil particles to disperse, decreasing the rate of infiltration and leaching. Additionally, the use of distilled water may lead to the removal of sorbed ions from soil particles, thereby affecting the degree of adsorption of the test material.

E. The soil treatment rate was not equal to the highest recommended rate for a single application. The fortification rate used in this study was equivalent to 0.1 ppm. Therefore, it was lower than the lowest proposed application rate. The lowest proposed application rate is 0.125 lb a.i./acre, applied three times per season, for peanuts and the highest is 0.825 lb a.i./acre, applied twice per season, for apples.

F. The soil moisture content was not adjusted to 75% of 1/3 bar prior to the pre-incubation period. The soil moisture was adjusted to approximately 40% of the maximum water-holding capacity (p. 26). The study author did not report the relationship between the two moisture contents for the soil utilized in the study.

G. The bulk densities of the packed soils were not reported. Soil bulk densities should be similar for all columns (hand packed) of the same soil.

H. Only foreign soils were utilized in this study. The study author classified the loam and the loamy sand soils (USDA particle-size classification) as a clay loam soil and a sandy loam soil, respectively (p. 13). It could not be determined whether the soils were classified according to the USDA soil taxonomic classification system. The author should provide clarification according to the USDA soil taxonomic classification. The soil may be not representative of the soils in the typical peanuts/apple growing areas of the United States.

I. All data were reported incorrectly with respect to depth of leaching. The top of the column was designated as 30 cm, while the bottom was designated as 0 cm. Generally, "0" indicates the top of the column and the values (cm) increase with depth.

J. The study author stated that the limits of detection were derived statistically from background counts (p. 24); however, the limits of detection and quantitation were not reported. It is necessary that both limits of detection and quantitation be reported.

REFERENCES

Helling. C.S., 1971. Pesticide mobility of soils II., Application of soil thin layer chromatography. *Soil Sci. Soc. Amer*, Proc., **35**, pp 737-743.

McCall P.J., Swann R.L., Laskowski D.A., Unger S.M., Vrona S. A. and Dishburger H.J., 1980 Estimation of Chemical Mobility in Soil from Liquid chromatographic Retention Times. Bull. *Environ. Contam. Toxicol.* **24**, pp190-195.

Prohexadione Calcium

DER MRID 44457788

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